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(57) Abstract

The invention relates to a method for controlling lice on an organism, comprising of treating the organism with a composition at least consisting of: a) lactoperoxidase; b) thiocyanate; and/or c) iodide; and d) a hydrogen peroxide source. The method is particularly suitable for controlling sea lice in fish or crustaceans, but can also be applied against aphids on plants and head lice on humans. The invention further relates to a composition and kit for use in the method.

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METHOD AND COMPOSITION FOR CONTROLLING LICE

The present invention relates to a method and device for controlling lice, in particular sea lice.

Sea louse is the general name of parasitic oar-footed crustaceans (copepods), which are found at (marine) water culture production locations where fish are farmed. In Northern Europe and particularly in Scotland and Norway sea lice are already the most significant threat to production locations of sea fish and the problem only continues to grow. Damage and death of fish due to sea louse infections are an important cost overhead in aquaculture. The problem also occurs in seawater and brackish water fish farms and shrimp ponds in South-East Asia, particularly in Thailand, the Philippines and Indonesia.

The parasitic sea louse copepods belong to the family of the Caligidae, comprising 23 genera and 200 species. The most important genera are Lepeophteirus, Caligus and Pseudocaligus, because they can result in high mortality. In Northern Europe the most important parasite is Lepeophtheirus salmonis and to a lesser degree Caligus elongatus. Both belong to the Caligidae and are ectoparasites on salmonoids. The life cycle of L.salmonis comprises 10 stages, of which the 4 chalimus stages infect the salmon. They attach themselves to the fish with claw-shaped antennae, penetrate the skin of the fish and thus cause skin lesions and large open wounds. Secondary bacterial and fungal infections are subsequently often the cause of death of the fish.

In order to control the sea lice use is often made [illegible] quantity of about [illegible] mg [illegible] substance [illegible] 5,313,911 in the name of Eka Nobel AB). However, both the large volumes of hydrogen peroxide and the limited activity and toxicity for the fish do not make this an ideal method.

Bath treatments are further also applied with different types of pesticide such as Cypermethrin™, Nuvan™ (active substance dichlorvos), Pyrethrum™ and Dipterex™ (active substance trichlorophon). These substances can only be used under heavy restrictions and have great drawbacks. They are not only toxic for the lice, but also harmful to the fish and the environment. Residues of the substances moreover accumulate in the fish and thus form an indirect risk to the consumer. Handling of the substances also involves risks. Finally, these substances are not active against every stage of development of the sea lice.

In the light of the above, it is the object of the present invention to provide an effective, natural and environmentally-friendly system with which the lice can be controlled without too many drawbacks for the fish.

This is achieved by the invention with a method for controlling lice on an organism, comprising of treating the organism with a composition which at least consists of:

a) lactoperoxidase;
b) thiocyanate; and/or
c) iodide; and
d) a hydrogen peroxide source.

Although this method is particularly suitable for controlling sea lice, it can also be employed to control other lice, such as aphids on plants, lice on animals, such as head lice in people.

The methods of administration in controlling other types of lice are of course not the same as in controlling sea lice. In the latter case the agent is added to water in which the fish are accommodated for a longer or shorter time or in which they are immersed. Controlling lice on plants can for instance be done by spraying, while treatment of head louse can take place by rinsing, rubbing-in or spraying.

The composition consisting of lactoperoxidase, thiocyanate and or iodide and hydrogen peroxide is most

effective when the concentration thereof with which the lice come into contact amounts for lactoperoxidase (LP) to 0.5 to 20 mg/l, preferably 1 to 10 mg/l, more preferably 2.5 to 7.5 mg/l and most preferably about 5 mg/l, for hydrogen peroxide to at least 10, preferably at least 50, more preferably at least 100 mg/l, for thiocyanate (SCN^-) to at least about 2.5 mg/l, preferably at least about 5 mg/l, more preferably at least about 10 mg/l, but a maximum of 100 mg/l, and for iodide (I^-) to at least about 5 mg/l, preferably at least about 30 mg/l, but a maximum of 100 mg/l.

When reference is made in this application to "concentration with which the lice come into contact", this is intended to mean the concentration which is present in the treatment bath in the case of sea lice, or in the spray or other means of application in the case of other lice. "Composition" is likewise understood to mean that in which the concentrations are equal to the treatment concentrations.

Such a composition for controlling lice on an organism therefore comprises the following active substances:

a) lactoperoxidase (LP) in a quantity of 0.5 to 20 mg/l, preferably 1 to 10 mg/l, more preferably 2.5 to 7.5 mg/l and most preferably about 5 mg/l;

b) hydrogen peroxide in a quantity of at least 10, preferably at least 50, more preferably at least 100 mg/l;

c) thiocyanate (SCN^-) in a quantity of at least about 2.5 mg/l, preferably at least about 5 mg/l, more preferably at least about 10 mg/l, but a maximum of 100 mg/l; and

d) iodide (I^-) in a quantity of at least about [illegible] mg/l, preferably at least about [illegible] mg/l, but a maximum of 100 mg/l. In a preferred embodiment the composition according to the invention comprises: 5 mg/l lactoperoxidase, 10 mg/l thiocyanate, 30 mg/l iodide and [illegible] mg/l hydrogen peroxide.

This composition can in turn be manufactured from a kit consisting of at least two components.

The components of the kit are at least two components, wherein the first component comprises lactoperoxidase, thiocyanate and/or iodide and the second component hydrogen peroxide. Supplying hydrogen peroxide separately prevents lactoperoxidase already becoming active in the packaging. The activity of the final composition could thereby be reduced. A kit may however also consist of more than two components, wherein in addition to the separate hydrogen peroxide the other constituents are also supplied separately or in pairs of two. The components can each individually be in liquid or solid form. Particularly the component consisting of lactoperoxidase, thiocyanate and/or iodide also forms part of the invention.

A particularly advantageous embodiment of the kit comprises at least two components, wherein the first component is formed by a composition comprising lactoperoxidase in a quantity resulting in a concentration with which the lice come into contact of 0.5 to 20 mg/l, preferably 1 to 10 mg/l, more preferably 2.5 to 7.5 mg/l and most preferably about 5 mg/l, thiocyanate in a quantity resulting in a concentration with which the lice come into contact of at least about 2.5 mg/l, preferably at least about 5 mg/l, more preferably at least about 10 mg/l, but a maximum of 100 mg/l, and/or iodide in a quantity resulting in a concentration with which the lice come into contact of at least about 5 mg/l, preferably at least about 20 mg/l, but a maximum of 100 mg/l, and the second component is formed by a solution of hydrogen peroxide in a quantity resulting in a concentration with which the lice come into contact of at least about 10 mg/l, preferably at least about 50 mg/l, more preferably at least 100 mg/l. Particularly recommended is a kit wherein the first component consists of lactoperoxidase in a quantity resulting in a concentration with which the lice come into contact of

about 5 mg/l, thiocyanate in a quantity resulting in a concentration with which the lice come into contact of about 10 mg/l and/or iodide in a quantity resulting in a concentration with which the lice come into contact of about 30 mg/l, and the second component is formed by a solution of hydrogen peroxide in a quantity resulting in a concentration with which the lice come into contact of 100 mg/l.

The composition can also be formed from a concentrated composition or a solid composition which contains all constituents. The desired treatment concentrations then result by adding this concentrated liquid or solid composition to water.

It is recommended to prepare the treatment bath prior to the fish being placed therein. This prevents damage to the fish occurring due to locally high concentrations of the different constituents which have not yet dissolved or are not yet well distributed through the water.

In order to safeguard the health of the fish as much as possible, the treatment time is preferably kept as short as possible. Treatment times between 5 and 60 minutes are recommended. Although the agent according to the invention is much less toxic than the high concentrations of hydrogen peroxide or pesticides used heretofore, it is nevertheless recommended to limit the contact between fish and active substances as much as possible. The treatment may optionally be repeated at a later stage in the case control is not complete.

The invention further relates to the use of the composition, kit or components thereof for controlling lice in general and sea lice in particular. The invention also comprises the use of the kit or the components thereof for manufacturing the composition.

The invention will be further illustrated with reference to the examples following below. The composition according to the invention is herein designated as "LI system".

EXAMPLES

EXAMPLE 1

In vitro tests with lactoperoxidase (LP) systems against sea louse

Egg sacs of the sea louse Lepeophteirus salmonis were collected and incubated for 5 days at 15°C in water with a salinity of 3.4% per weight. During this time the oar-footed crustaceans developed into healthy specimens.

One or more of the components lactoperoxidase, iodide, thiocyanate and hydrogen peroxide were dissolved in seawater sterilized using an ozone treatment and filtration. About 200 copepodids per litre were subsequently added to each of these solutions and incubated for 20 minutes. The copepodids were then filtered off, washed and placed once again in clean seawater. The percentage of surviving copepodids was determined after 1 hour.

The composition of the solutions and the survival of the copepodids therein are shown in tables 1 and 2.

Table 1 Effect of individual components

	Blank	I^- Only	SCN^- Only	H_2O_2 Only	LP Only	LP Only
Lactoperoxidase, mg/l	0	0	0	0	10	20
K-iodide, mg/l	0	30	0	0	0	0
K-thiocyanate, mg/l	0	0	10	0	0	0
H_2O_2, mg/l	0	0	0	200	0	0
% survival after 1 hour	95	88	86	93	90	69

Table 2 Effect of LP systems

Lactoperoxidase, mg/l	0	2.5	5	10	10	10	20	20
K-iodide, mg/l	0	30	30	30	30	30	30	30
K-thiocyanate, mg/l	0	10	10	10	10	10	10	10
H_2O_2, mg/l	0	100	100	10	50	100	100	200
% survival after 1 hour	95	73	39	31	6	0	0	0

From tables 1 and 2 can be seen that the individual components have hardly any effect on the sea lice, but that the combination thereof in the LP system according to the invention does so.

EXAMPLE 2

Sensitivity of fish to LP systems

Young salmon with an average weight of about 50 g. were exposed to solutions (in seawater) of the individual components and to complete LP systems. The table below shows the concentration.

components group	control	1	2	3	4	5	6
Lactoperoxidase mg/l	0	10	0	0	5	2.5	1
K-thiocyanate mg/l	0	0	10	0	5	2.5	2.5
K-iodide mg/l	0	0	0	30	5	7.5	7.5
H_2O_2, mg/l	0	0	0	0	100	100	100

The transfer of young salmon to a new environment will in any case cause stress phenomena, such as a slightly increased gill cover activity and agitated swimming on the surface. Calm is virtually restored after about 30 minutes.

These phenomena were observed in both the control and solutions of the individual components, wherein there was hardly any difference, or none at all, between the control and individual components.

The stress reactions with complete LP systems were clearly higher, at the lowest concentration (1 mg/l LP) agitated swimming behaviour and a moderately increased gill cover activity was still present after 30 minutes. Only after 60 minutes was calm restored.

At a concentration of 2.5 mg/l LP these phenomena were more pronounced but still acceptable.

At a concentration of 5 ppm LP stress phenomena were even more severe. After 60 minutes the majority of the fish was still lethargic and swimming in uncoordinated manner. There were no fatalities however.

A test with larger salmon (about 500 g) and a system with 5 mg/l LP showed that these fish were hardly affected by the treatment.

EXAMPLE

In vivo study of the effect of LP systems on fish which "infected" with sea lice

In this example the effect of an LP system with 2.5 mg/l LP, 2.5 mg/l KSCN, 7.5 mg/l KI and 100 mg/l H_2O_2 is

studied in a situation in which young Atlantic salmon were "infected" with sea lice of L. salmonis.

1. Method

In four tanks of 1m^3 each, 40 young salmon of about 50g in their second year of life were kept per tank in seawater treated with ozone (>750 Mv) and filtered by carbon at ambient temperature (14°C ± 1°C). Per tank the fish were brought into contact with 1000 copepodids of L. salmonis for 1.5 hours and the lice were allowed to develop to pre-adult stages.

Counts were carried out prior to the treatment and it was determined that all tanks contained sufficient pre-adult stage lice. The treatments were then started.

Two of the tanks (1 and 3) were treated with the LP system according to the invention (2.5 mg/l LP, 2.5 mg/l KSCN, 7.5 mg/l KI and 100 mg/l H_2O_2) for 20 minutes. Tanks 2 and 4 received an identical treatment with seawater. The temperature of the seawater was 15°C and it contained more than 8 mg/l oxygen. Samples were assessed 1, 24 and 48 hours after treatment. Lice levels on the fish were recorded and compared with the levels before the treatment making use of Student's t-test.

2. Results

The results of the lice counts are shown in the table below. The lice counts are expressed per fish and are average values of 10 fish.

Table 9

Sea lice counts: average values per fish (n=10)

	before treatment	after treatment, 1 hour	after treatment, 24 hours	after treatment, 48 hours
Tank 1 (LP-s)	4.0 (SEM = 0.494)	3.3 (SEM = 0.60)	2.5 (SEM = 0.64)	1.5 (SEM = 0.5)
Tank 2 (control)	4.8 (SEM = 0.629)	4.4 (SEM = 0.56)	4.2 (SEM = 0.61)	3.4 (SEM = 0.4)
Tank 3 (LP-s)	4.7 (SEM = 0.731)	4.0 (SEM = 0.56)	2.1 (SEM = 0.41)	2.2 (SEM = 0.2)
Tank 4 (control)	3.5 (SEM = 0.401)	5.1 (SEM = 0.74)	3.7 (SEM = 0.94)	3.8 (SEM = 1.08)

Lice levels

1 hour after the treatment there was no significant reduction in lice levels in any of the groups. After 24 hours both treated groups had significantly fewer lice. 48 hours after the treatment there was a reduction of respectively 63% and 53% ($p<0.01$). There was no significant reduction in lice levels in untreated control groups.

Behaviour of the fish

In the eleventh minute during the treatment the fish displayed some agitation with an increase to rapid swimming and jumping activity at 15 minutes. At 19 minutes some fish were at the point of death and only recovered after the tank had been flushed. There were no fatalities.

The treatment with an LP system consisting of 2.5 mg/l LP, 2.5 mg/l KSCN, 7.5 mg/l KI and 100 mg/l H_2O_2 for 20 minutes was on average 58% effective against the sea lice. There appeared to be some effect on the fish, but this was not fatal. Because small fish were treated here at a high seawater temperature, this test was performed under the worst possible conditions. Larger fish at a lower temperature will be more resistant to the effects of an LP system.

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REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

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Methods and composition for controlling lice

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Box No. VI PRIORITY CLAIM ☐ Further priority claims are indicated in the Supplemental Box

Filing date of earlier application (day/month/year)	Number of earlier application	Where earlier application is: national application country	regional application * regional Office	international application receiving Office
item (1) (26.03.99) 26 March 1999	1011681	NL		
item (2)				
item (3)				

☒ The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) *(only if the earlier application was filed with the Office which for the purposes of the present international application is the receiving Office)* identified above as item(s): 1

* *Where the earlier application is an ARIPO application, it is mandatory to indicate in the Supplemental Box at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed (Rule 4.10(b)(ii))* See Supplemental Box

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) *(if two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen, the two-letter code may be used):* ISA/EPO

Request to use results of earlier search; reference to that search *(if an earlier search has been carried out by or requested from the International Searching Authority)*

Date (day/month/year)	Number	Country (or regional Office)
26 March 1999	SN 32816	NL

Box No. VIII CHECK LIST; LANGUAGE OF FILING

This international application contains the following **number of sheets**:

request	4
description (excluding sequence listing part)	11
claims	4
abstract	1
drawings	
sequence listing part of description	
Total number of sheets	20

This international application is **accompanied by** the item(s) marked below:

1. ☒ fee calculation sheet
2. ☐ separate signed power of attorney
3. ☐ copy of general power of attorney; reference number, if any:
4. ☐ statement explaining lack of signature
5. ☐ priority document(s) identified in Box No. VI as item(s):
6. ☐ translation of international application into (language):
7. ☐ separate indications concerning deposited microorganism or other biological material
8. ☐ nucleotide and/or amino acid sequence listing in computer readable form
9. ☐ other *(specify)*:

Figure of the drawings which should accompany the abstract:

Language of filing of the international application: Dutch

Box No. IX SIGNATURE OF APPLICANT OR AGENT

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

Van Someren, Petronella Francisca Hendrika Maria

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1. Date of actual receipt of the purported international application: 23 MAR 2000 (23.03.00)
2. Drawings: ☐ ... ☐ not received
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:
4. Date of timely receipt of the required corrections under PCT Article 11(2):
5. International Searching Authority (if two or more are competent): ISA/
6. ☐ Transmittal of search copy delayed until search fee is paid

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Date of receipt of the record copy by the International Bureau: 19 APRIL 2000 (19.04.00)

WERKWIJZE EN SAMENSTELLING VOOR HET BESTRIJDEN VAN LUIZEN

De onderhavige uitvinding heeft betrekking op een werkwijze en inrichting voor het bestrijden van luizen, in het bijzonder zeeluizen.

Zeeluis is de algemene naam van parasitaire roeipootkreeften (copepoden), die worden gevonden op (marine) watercultuur productielocaties, waar vissen worden gekweekt. In Noord-Europa en met name Schotland en Noorwegen zijn zeeluizen reeds de belangrijkste bedreiging voor productielocaties van zeevissen en het probleem neemt alleen nog maar toe. Schade en sterfte van de vis door zeeluisinfecties zijn een belangrijke kostenpost in de aquacultuur. Het probleem doet zich eveneens voor zee- en brakwaterviskwekerijen en garnalenvijvers in Zuidoost-Azië, met name in Thailand, de Filipijnen en Indonesië.

De parasitaire zeeluiscopepoden behoren tot de familie van de Caligidae, die 23 genera en 200 species omvat. De belangrijkste genera zijn Lepeophteirus, Caligus en Pseudocaligus, omdat zij kunnen leiden tot hoge sterfte. In Noord Europa is de belangrijkste parasiet Lepeophtheirus salmonis en in minder mate Caligus elongatus. Beiden behoren tot de Caligidae en zijn ectoparasieten op zalmachtigen. De levenscyclus van L.salmonis omvat 10 stadia, waarvan de 4 chalimusstadia de zalm infecteren. Zij klemmen zich aan de vis vast met klauwvormige antennes, penetreren de huid van de vis en veroorzaken zo huidlesies en grote open wonden. Secundaire bacteriële en schimmelinfecties zijn vervolgens vaak de oorzaak van de sterfte van de vis.

Om de zeeluizen te bestrijden wordt vaak gebruik gemaakt van waterstofperoxide, dat in een hoeveelheid van ongeveer 1500 mg/l [illegible] wordt toegevoegd. Dit is bijvoorbeeld [illegible] van Eka Nobel AB). Echter, zowel de grote volumina waterstofperoxide, als de beperkte werkzaamheid en toxiciteit voor de vissen maken dit geen ideale methode.

Verder worden ook badbehandelingen met verschillende soorten pesticiden toegepast, zoals Cypermetrin™, Nuvan™ (actieve stof dichloorvos), Pyrethrum™ en Dipterex™ (actieve stof trichloorphon). Deze stoffen kunnen slechts onder zware restricties worden gebruikt en hebben grote nadelen. Ze zijn niet alleen toxisch voor de luizen, maar ook schadelijk voor de vis en het milieu. Bovendien hopen resten van de stoffen zich op in de vis en vormen zo indirect een risico voor de consument. Ook het hanteren van de stoffen brengt risico's met zich mee. Tenslotte zijn deze stoffen niet tegen elk ontwikkelingsstadium van de zeeluizen werkzaam.

In het licht van het bovenstaande is het het doel van de onderhavige uitvinding een effectief, natuurlijk en milieuvriendelijk systeem te verschaffen, waarmee de luizen zonder al te veel nadelen voor de vis kunnen worden bestreden.

Dit wordt door de uitvinding bereikt door een werkwijze voor het bestrijden van luizen op een organisme, omvattende het behandelen van het organisme met een samenstelling, die ten minste bestaat uit:

a) lactoperoxidase;
b) thiocyanaat; en/of
c) jodide; en
d) een waterstofperoxide-bron.

Hoewel deze werkwijze bijzonder geschikt is voor de bestrijding van zeeluizen kan hij ook worden ingezet voor de bestrijding van andere luizen, zoals bladluizen op planten, als luizen op dieren, zoals hoofdluizen bij mensen.

De toedieningswijzen zijn bij de bestrijding van andere soorten luizen uiteraard niet hetzelfde als bij de bestrijding van zeeluizen. In het laatste geval worden de middelen toegevoegd aan water waarin de vissen voor langere of kortere tijd verblijven of waarin ze worden ondergedompeld. Bestrijding van luizen op planten kan bijvoorbeeld door sproeien, terwijl behandeling van

hoofdluis kan plaatsvinden door spoelen, insmeren, of sproeien.

De samenstelling, die bestaat uit lactoperoxidase, thiocyanaat en/of jodide en waterstofperoxide is het meest effectief wanneer de concentratie daarvan waarmee de luizen in contact komen voor lactoperoxidase (LP) 0,5 tot 20 mg/l, bij voorkeur 1 tot 10 mg/l, meer bij voorkeur 2,5 tot 7,5 mg/l en meest bij voorkeur ongeveer 5 mg/l bedraagt, voor waterstofperoxide ten minste 10, bij voorkeur tenminste 50, meer bij voorkeur tenminste 100 mg/l bedraagt, voor thiocyanaat (SCN^-) ten minste ongeveer 2,5 mg/l, bij voorkeur ten minste ongeveer 5 mg/l, meer bij voorkeur ten minste ongeveer 10 mg/l, maar maximaal 100 mg/l bedraagt en voor jodide (I^-) ten minste ongeveer 5 mg/l, bij voorkeur ten minste ongeveer 30 mg/l, maar maximaal 100 mg/l bedraagt.

Wanneer in deze aanvrage wordt gesproken over "concentratie waarmee de luizen in contact komen" wordt daarmee de concentratie bedoeld zoals die aanwezig is in het behandelbad, in geval van zeeluizen of in de spray, of andere toedieningsvorm in geval van ander luizen. Met "samenstelling" wordt eveneens datgene bedoeld, waarin de concentraties gelijk zijn aan de behandelconcentraties.

Een dergelijke samenstelling voor het bestrijden van luizen op een organisme, omvat derhalve de volgende actieve stoffen:

a) lactoperoxidase (LP) in een hoeveelheid van 0,5 tot 20 mg/l, bij voorkeur 1 tot 10 mg/l, meer bij voorkeur 2,5 tot 7,5 mg/l en meest bij voorkeur ongeveer 5 mg/l;

b) waterstofperoxide in een hoeveelheid van ten minste 10, bij voorkeur tenminste 50, meer bij voorkeur tenminste 100 mg/l;

c) thiocyanaat (SCN^-) in een hoeveelheid van ten minste ongeveer 2,5 mg/l, bij voorkeur ten minste ongeveer 5 mg/l, meer bij voorkeur ten minste ongeveer 10 mg/l, maar maximaal 100 mg/l; en

d) jodide (I^-) in een hoeveelheid van ten minste ongeveer 5 mg/l, bij voorkeur ten minste ongeveer 20 mg/l, maar maximaal 100 mg/l. In een voorkeursuitvoeringsvorm omvat de samenstelling volgens de uitvinding: 5 mg/l lactoperoxidase, 10 mg/l thiocyanaat, 30 mg/l jodide en 100 mg/l waterstofperoxide.

Deze samenstelling kan op zijn beurt worden vervaardigd uit een kit, die bestaat uit tenminste twee componenten.

De componenten uit de kit zijn tenminste twee componenten, waarbij de eerste component lactoperoxidase, thiocyanaat en/of jodide omvat en de tweede component waterstofperoxide. Het apart leveren van waterstofperoxide voorkomt dat lactoperoxidase reeds in de verpakking actief wordt. De activiteit van de uiteindelijke samenstelling zou daardoor kunnen verminderen. Een kit kan echter ook bestaan uit meer dan twee componenten, waarbij naast het aparte waterstofperoxide ook de andere bestanddelen apart of in paren van twee worden geleverd. De componenten kunnen elk afzonderlijk in vloeibare of vaste vorm zijn. Met name de component die bestaat uit lactoperoxidase, thiocyanaat en/of jodide maakt eveneens onderdeel uit van de uitvinding.

In een bijzonder voordelige uitvoeringsvorm van de kit omvat deze ten minste een tweetal componenten, waarbij de eerste component wordt gevormd door een samenstelling omvattende lactoperoxidase in een hoeveelheid die resulteert in een concentratie, waarmee de luizen in contact komen van 0,5 tot 20 mg/l, bij voorkeur 1 tot 10 mg/l, meer bij voorkeur 2,5 tot 7,5 mg/l en meest bij voorkeur ongeveer 5 mg/l, thiocyanaat in een hoeveelheid die resulteert in een concentratie, waarmee de luizen in contact komen van ten minste ongeveer 5 mg/l, bij voorkeur ten minste ongeveer 5 mg/l, meer bij voorkeur ten minste ongeveer 10 mg/l, maar maximaal 100 mg/l en/of jodide in een hoeveelheid die resulteert in een concentratie, waarmee de luizen in contact komen van ten minste

ongeveer 5 mg/l, bij voorkeur ten minste ongeveer 20 mg/l, maar maximaal 100 mg/l, en de tweede component wordt gevormd door een oplossing van waterstofperoxide in een hoeveelheid die resulteert in een concentratie, waarmee de luizen in contact komen van ten minste 10, bij voorkeur tenminste 50, meer bij voorkeur tenminste 100 mg/l. De bijzondere voorkeur gaat uit naar een kit waarbij de eerste component bestaat uit lactoperoxidase in een hoeveelheid die resulteert in een concentratie, waarmee de luizen in contact komen van ongeveer 5 mg/l, thiocyanaat in een hoeveelheid die resulteert in een concentratie, waarmee de luizen in contact komen van ongeveer 10 mg/l en/of jodide in een hoeveelheid die resulteert in een concentratie, waarmee de luizen in contact komen van ongeveer 30 mg/l, en de tweede component wordt gevormd door een oplossing van waterstofperoxide in een hoeveelheid die resulteert in een concentratie, waarmee de luizen in contact komen van 100 mg/l.

De samenstelling kan ook worden gevormd uit een geconcentreerde samenstelling of een vaste samenstelling, die alle bestanddelen bevat. Door deze geconcentreerde vloeibare of vaste samenstelling toe te voegen aan water ontstaan dan de gewenste behandelconcentraties.

Het heeft de voorkeur om het behandelbad klaar te maken voordat de vissen daarin worden uitgezet. Dit voorkomt dat door plaatselijk hoge concentraties van de verschillende, nog niet opgeloste of nog niet goed door het water verdeelde bestanddelen schade aan de vissen optreedt.

Om de gezondheid van de vissen zoveel mogelijk te sparen wordt de behandeltijd liefst zo kort mogelijk gehouden. Behandeltijden tussen 5 en 60 minuten hebben de

[illegible] gebruikte hoge concentraties waterstofperoxide of pesticiden, verdient het toch de voorkeur het contact tussen vissen en actieve stoffen zo veel mogelijk te beperken. Eventueel kan bij

een niet volledige bestrijding de behandeling in een later stadium worden herhaald.

De uitvinding heeft verder betrekking op het gebruik van de samenstelling, kit of componenten daarvan voor het bestrijden van luizen in het algemeen en zeeluizen in het bijzonder. Ook wordt door de uitvinding omvat het gebruik van de kit of de componenten daaruit voor het vervaardigen van de samenstelling.

De uitvinding zal verder worden geïllustreerd aan de hand van de hierna volgende voorbeelden. De samenstelling volgens de uitvinding wordt hierin aangeduid als "LP-systeem".

VOORBEELDEN

VOORBEELD 1

In vitro proeven met lactoperoxidase (LP)-systemen tegen zeeluis

Eierzakjes van de zeeluis Lepeophteirus salmonis werden verzameld en gedurende 5 dagen bij 15°C geïncubeerd in water met een zoutgehalte van 3,4 gew.%. In die tijd ontwikkelden de roeipootkreeften zich tot gezonde exemplaren.

Eén of meer van de componenten lactoperoxidase, jodide, thiocyanaat en waterstofperoxide werden opgelost in met behulp van een ozonbehandeling en filtratie gesteriliseerd zeewater. Vervolgens werden aan elk van deze oplossingen per liter ongeveer 200 copepodiden toegevoegd en 20 minuten geïncubeerd. De copepodiden werden daarna afgefiltreerd, gewassen en opnieuw in schoon zeewater geplaatst. Na 1 uur werd het percentage overlevende copepodiden bepaald.

De samenstelling van de copepodiden daarna is weergegeven in tabellen 1 en 2.

Tabel 1 Effect afzonderlijke componenten

	Blanco	Alleen I^-	Alleen SCN^-	Alleen H_2O_2	Alleen LP	Alleen LP
Lactoperoxidase, mg/l	0	0	0	0	10	20
K-jodide, mg/l	0	30	0	0	0	0
K-thiocyanaat, mg/l	0	0	10	0	0	0
H_2O_2, mg/l	0	0	0	200	0	0
% overleving na 1 uur	95	88	86	93	90	69

Tabel 2 Effect LP-systemen

Lactoperoxidase,mg/l	0	2,5	5	10	10	10	20	20
K-jodide, mg/l	0	30	30	30	30	30	30	30
K-thiocyanaat, mg/l	0	10	10	10	10	10	10	10
H_2O_2, mg/l	0	100	100	10	50	100	100	200
% overleving na 1 uur	95	73	39	31	6	0	0	0

Uit de tabellen 1 en 2 blijkt dat de afzonderlijke componenten nauwelijks enig effect hebben op de zeeluizen, maar de combinatie daarvan in het LP-systeem volgens de uitvinding wel.

VOORBEELD 2

Gevoeligheid van vissen voor LP-systemen

Jonge zalmen met een gemiddeld gewicht van ca 50 g. werden blootgesteld aan oplossingen (in zeewater) van de afzonderlijke componenten en aan complete LP-systemen.

componenten groep	controle	1	2	3	4	5	6
Lactoperoxidase mg/l	0	10	0	0	5	2,5	1
K-thiocyanaat mg/l	0	0	10	0	5	2,5	2,5
K-jodide mg/l	0	0	0	30	5	7,5	7,5
H_2O_2, mg/l	0	0	0	0	100	100	100

Overbrengen van jonge zalmen naar een nieuwe omgeving geeft sowieso aanleiding tot stressverschijnselen, zoals een enigszins verhoogde kieuwdekselactiviteit en geagiteerd zwemmen aan het oppervlak. Na ca. 30 minuten is de rust vrijwel teruggekeerd.

Zowel in de controle als in oplossingen van de afzonderlijke componenten werden deze verschijnselen waargenomen waarbij nauwelijks of geen verschil was tussen de controle en afzonderlijke componenten.

De stressreacties met complete LP-systemen waren duidelijk hoger, bij de laagste concentratie (1 mg/l LP), was na 30 minuten nog steeds geagiteerd zwemgedrag en een matig verhoogde kieuwdekselactiviteit. Pas na 60 minuten was de rust teruggekeerd.

Bij een concentratie van 2.5 mg/l LP waren deze verschijnselen uitgesprokener maar nog steeds aanvaardbaar.

Bij een concentratie van 5 ppm LP waren stress-verschijnselen nog heviger. Na 60 minuten was de meerderheid van de vissen nog lethargisch en zwom ongecoördineerd. Er waren echter geen sterfgevallen.

Een proef met grotere zalmen (ca. 500 g) en een systeem met 5 mg/l LP gaf aan dat deze vissen nauwelijks

VOORBEELD 3

In vivo studie van het effect van LP-systemen bij vissen die "besmet" worden door zeeluizen

In dit voorbeeld wordt het effect van een LP-systeem met 2,5 mg/l LP, 2,5 mg/l KSCN, 7,5 mg/l KI en 100 mg/l H_2O_2 bestudeerd in een situatie waarin jonge atlantische zalmen "besmet" werden met zeeluizen van L. salmonis.

1. Methode

In vier tanks van 1m³ elk werden per tank 40 jonge zalmen van ongeveer 50g in hun tweede levensjaar gehouden in met ozon behandeld (>750 Mv), door koolstof gefilterd zeewater bij omgevingstemperatuur (14°C ± 1°C). Per tank werden de vissen gedurende 1,5 uur in contact gebracht met 1000 copepodiden van L.salmonis en men liet de luizen zich ontwikkelen tot pre-adult stadia.

Voorafgaand aan de behandeling werden tellingen uitgevoerd en vastgesteld dat alle tanks voldoende pre-adult stadium luizen bevatten. Vervolgens werden de behandelingen gestart.

Twee van de tanks (1 en 3) werden behandeld met het LP-systeem volgens de uitvinding (2,5 mg/l LP, 2,5 mg/l KSCN, 7,5 mg/l KI en 100 mg/l H_2O_2) gedurende 20 minuten. Tanks 2 en 4 ontvingen een identieke behandeling met zeewater. De temperatuur van het zeewater was 15°C en het bevatte meer dan 8 mg/l zuurstof. Monsters werden beoordeeld op 1, 24 en 48 uur na behandeling. Luizenniveaus op de vissen werden opgetekend en vergeleken met de niveaus vóór de behandeling met gebruikmaking van student's t test.

2. Resultaten

De resultaten van de luizentellingen worden weergegeven in de onderstaande tabel. De luizentellingen worden uitgedrukt per vis en zijn gemiddelde waarden van 10 vissen.

Tabel 9

Zeeluizentellingen: gemiddelde waarden per vis (n=10)

	voor behandeling	na behandeling, 1 uur	na behandeling, 24 uur	na behandeling, 48 uur
Tank 1 (LP-s)	4.0 (SEM =0.494)	3.3 (SEM =0.60)	2.5 (SEM =0.64)	1.5 (SEM =0.5)
Tank 2 (controle)	4.8 (SEM =0.629)	4.4 (SEM =0.56)	4.2 (SEM =0.61)	3.4 (SEM =0.4)
Tank 3 (LP-s)	4.7 (SEM =0.731)	4.0 (SEM =0.56)	2.1 (SEM =0.41)	2.2 (SEM =0.2)
Tank 4 (controle)	3.5 (SEM =0.401)	5.1 (SEM =0.74)	3.7 (SEM =0.94)	3.8 (SEM =1.08)

Luisniveaus

Op 1 uur na de behandeling was er in geen van de groepen een significante reductie in luizenniveaus. Na 24 uur hadden beide behandelde groepen significant minder luizen. 48 Uur na de behandeling was er een vermindering van respectievelijk 63% en 53% ($p<0,01$). Er was geen significante vermindering in luizenniveaus in onbehandelde controlegroepen.

Gedrag van de vissen

In de elfde minuut tijdens de behandeling vertoonde de vissen enige agitatie met een toename naar snel zwemmen en springactiviteit op 15 minuten. Op 19 minuten werden sommige vissen zieltogend en herstelden pas nadat de tank doorgespoeld was. Er waren geen sterfgevallen.

De behandeling met een LP-systeem bestaande uit 2,5 mg/l LP, 2,5 mg/l KSCN, 7,5 mg/l KI en 100 mg/l H_2O_2 gedurende 30 minuten was gemiddeld 58% werkzaam tegen de zeeluizen. Er leek enig effect op de vissen te zijn, maar

deze was niet fataal. Doordat hier kleine vissen bij een hoge zeewatertemperatuur behandeld werden is deze proef onder de slechts mogelijke omstandigheden uitgevoerd. Grotere vissen bij een lagere temperatuur zullen resistenter zijn tegen de effecten van een LP-systeem.

CONCLUSIES

1. Werkwijze voor het bestrijden van luizen op een organisme, omvattende het behandelen van het organisme met een samenstelling, die ten minste bestaat uit:

a) lactoperoxidase;

b) thiocyanaat; en/of

c) jodide; en

d) een waterstofperoxide-bron.

2. Werkwijze volgens conclusie 1, **met het kenmerk**, dat de waterstofperoxide-bron waterstofperoxide zelf is of een systeem van glucose-oxidase en glucose, waardoor waterstofperoxide gegenereerd kan worden.

3. Werkwijze volgens conclusie 1 en 2, **met het kenmerk**, dat de luizen zeeluizen zijn en het organisme een vis of schaaldier is.

4. Werkwijze volgens conclusie 3, **met het kenmerk**, dat de samenstelling wordt toegevoegd aan het water waarin de vissen gehouden worden.

4. Werkwijze volgens conclusie 1 en 2, **met het kenmerk**, dat de luizen bladluizen zijn en het organisme een plant is.

5. Werkwijze volgens conclusie 1 en 2, **met het kenmerk**, dat de luizen zich op een dier bevinden.

6. Werkwijze volgens conclusie 1 en 2, **met het kenmerk**, dat de luizen hoofdluizen zijn en het organisme een mens is.

7. Werkwijze volgens conclusies 1-6, **met het kenmerk**, dat de concentratie lactoperoxidase (LP) waarmee de luizen in contact komen 0,5 tot 20 mg/l, bij voorkeur 1 tot 10 mg/l, meer bij voorkeur 2,5 tot 7,5 mg/l en meest bij voorkeur ongeveer 5 mg/l bedraagt.

[illegible] **merk** [illegible] concentratie waterstofperoxide waarmee de luizen in contact komen ten minste 10, bij voorkeur tenminste 50, meer bij voorkeur tenminste 100 mg/l bedraagt.

9. Werkwijze volgens conclusies 1-8, **met het kenmerk**, dat de concentratie thiocyanaat (SCN^-) waarmee de luizen in contact komen ten minste ongeveer 2,5 mg/l, bij voorkeur ten minste ongeveer 5 mg/l, meer bij voorkeur ten minste ongeveer 10 mg/l, maar maximaal 100 mg/l bedraagt.

10. Werkwijze volgens conclusies 1-9, **met het kenmerk**, dat de concentratie jodide (I^-) waarmee de luizen in contact komen ten minste ongeveer 5 mg/l, bij voorkeur ten minste ongeveer 20 mg/l, maar maximaal 100 mg/l bedraagt.

11. Samenstelling voor het bestrijden van luizen op een organisme, omvattende:

a) lactoperoxidase (LP) in een hoeveelheid van 0,5 tot 20 mg/l, bij voorkeur 1 tot 10 mg/l, meer bij voorkeur 2,5 tot 7,5 mg/l en meest bij voorkeur ongeveer 5 mg/l;

b) waterstofperoxide in een hoeveelheid van ten minste 10, bij voorkeur tenminste 50, meer bij voorkeur tenminste 100 mg/l;

c) thiocyanaat (SCN^-) in een hoeveelheid van ten minste ongeveer 2,5 mg/l, bij voorkeur ten minste ongeveer 5 mg/l, meer bij voorkeur ten minste ongeveer 10 mg/l, maar maximaal 100 mg/l; en

d) jodide (I^-) in een hoeveelheid van ten minste ongeveer 5 mg/l, bij voorkeur ten minste ongeveer 20 mg/l, maar maximaal 100 mg/l,

waarbij alle hoeveelheden actieve stof refereren aan de concentratie van de actieve stof waarmee de luizen in contact komen.

12. Samenstelling volgens conclusie 11, omvattende: 50 mg/l lactoperoxidase, 10 mg/l thiocyanaat, 30 mg/l jodide en 100 mg/l waterstofperoxide.

13. Kit voor het bestrijden van luizen op een organisme, welke kit ten minste een tweetal componenten omvat, waarbij de eerste component wordt gevormd door een samenstelling omvattende lactoperoxidase in een hoeveelheid die resulteert in een concentratie, waarmee de

luizen in contact komen van 0,5 tot 20 mg/l, bij voorkeur 1 tot 10 mg/l, meer bij voorkeur 2,5 tot 7,5 mg/l en meest bij voorkeur ongeveer 5 mg/l, thiocyanaat in een hoeveelheid die resulteert in een concentratie, waarmee de luizen in contact komen van ten minste ongeveer 2,5 mg/l, bij voorkeur ten minste ongeveer 5 mg/l, meer bij voorkeur ten minste ongeveer 10 mg/l, maar maximaal 100 mg/l en/of jodide in een hoeveelheid die resulteert in een concentratie, waarmee de luizen in contact komen van ten minste ongeveer 5 mg/l, bij voorkeur ten minste ongeveer 30 mg/l, maar maximaal 100 mg/l, en de tweede component wordt gevormd door een oplossing van waterstofperoxide in een hoeveelheid die resulteert in een concentratie, waarmee de luizen in contact komen van ten minste 10, bij voorkeur tenminste 50, meer bij voorkeur tenminste 100 mg/l.

14. Kit volgens conclusie 12, **met het kenmerk**, dat de eerste component bestaat uit lactoperoxidase in een hoeveelheid die resulteert in een concentratie, waarmee de luizen in contact komen van ongeveer 5 mg/l, thiocyanaat in een hoeveelheid die resulteert in een concentratie, waarmee de luizen in contact komen van ongeveer 10 mg/l en/of jodide in een hoeveelheid die resulteert in een concentratie, waarmee de luizen in contact komen van ongeveer 30 mg/l, en de tweede component wordt gevormd door een oplossing van waterstofperoxide in een hoeveelheid die resulteert in een concentratie, waarmee de luizen in contact komen van 100 mg/l.

15. Kit volgens conclusie 13 en 14, **met het kenmerk**, dat de eerste component een geconcentreerde vloeistof is.

16. Kit volgens conclusie 13 en 14, **met het kenmerk** dat de eerste component een [illegible]

component voor gebruik in een kit volgens conclusies 13-16, omvattende lactoperoxidase, thiocyanaat en/of jodide in een hoeveelheid die resulteert in een concentratie waarmee de luizen in contact komen als gegeven in conclusies 13 of 14.

18. Gebruik van een samenstelling volgens conclusies 11 en 12 voor de bestrijding van luizen op een organisme.

19. Gebruik van de kit volgens conclusies 13-16 voor het vervaardigen van een samenstelling volgens conclusies 11 of 12.

20. Gebruik van een component volgens conclusie 17 in een kit volgens conclusies 13-16.

UITTREKSEL

De uitvinding betreft een werkwijze voor het bestrijden van luizen op een organisme, omvattende het behandelen van het organisme met een samenstelling, die ten minste bestaat uit: a) lactoperoxidase; b) thiocyanaat; en/of c) jodide; en d) een waterstofperoxide-bron. De werkwijze is in het bijzonder geschikt voor de bestrijding van zeeluizen bij vissen of schaaldieren, maar kan ook worden toegepast tegen bladluizen op planten en hoofdluizen bij de mens. De uitvinding betreft verder een samenstelling en kit voor gebruik in de werkwijze.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
L/WZ42/cm/1		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/NL00/00196	23/03/2000	26/03/1999

International Patent Classification (IPC) or national classification and IPC
A01N63/00

Applicant
CAMPINA MELKUNIE B.V. et al.

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
25/10/2000	25.06.2001

Name and mailing address of the international preliminary examining authority:

European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx: 523656 epmu d
Fax: +49 89 2399 - 4465

Authorized officer
Faizi, R

Telephone No. +49 89 2399 8603

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/NL00/00196

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-11 as originally filed

Claims, No.:

1-20 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

   ☐ restricted the claims.

   ☐ paid additional fees.

   ☐ paid additional fees under protest.

   ☒ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

   ☐ complied with.

   ☐ not complied with for the following reasons:

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

   ☒ all parts.

   ☐ the parts relating to claims Nos. .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims 1-10
	No:	Claims 11-20
Inventive step (IS)	Yes:	Claims
	No:	Claims 1-20
Industrial applicability (IA)	Yes:	Claims 1-20
	No:	Claims

2. Citations and explanations
see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

IV: Unity:

The present Demand is directed to the control of "lice", this term being used to regroup organisms which are not only phylogenetically different from one another but which also differ in their habitats and life forms. Therefore, to extend a method of controlling sea lice which are crustaceans, to head lice which are anopluran insects and to aphids which are homopteran insects, cannot be found to involve one single invention. Since a completely different method will be involved for each type of "louse", (see also Applicant's statement at page 2, lines 29 to 32) and completely different compositions will be necessary for treating each locus, each group is considered as being distinct and to require different inventions.
Therefore, the requisite unity of invention (Rule 13.1 PCT) no longer exists inasmuch as a technical relationship in the meaning of Rule 13.2 PCT does not exist between the subject-matter of the following groups of dependent claims: 1 to 4 (1) , 7 to 10 (fish); 1, 4 (2) and 7 to 10 (aphids) , and 1, 5, 6 and 7 to 10 (animal and humans).

Applicant should inform the IPEA which invention or group of inventions he wishes to pursue further.

V: Reasoned Statements:

The claimed method is based on the use of a composition for controlling lice. This composition contains a) a lactoperoxidase,

b) a thiocyanate, and/or
c) an iodide
d) a hydrogen peroxide source.

Such a composition was described identically by D1: EP-A-0 307 376 which describes a microbicidal composition based on the above components see page 3, lines 1-15, D1. This composition can also be used in aquaculture, see page 3, line 18, D1. The relative amounts of the different components used by D1 are : lactoperoxidase: 0.2 mg/l,

ppm. These values correspond closely to those of the composition as disclosed in the present Demand.
Thus, having been identically described by D1, the subject-matter of the present Demand as defined by claims 11 to 20 lacks novelty.

D2: US-A-5 313 911 describes the use of hydrogen peroxide to control the salmon louse.

A skilled person, faced with the problem of controlling fish lice will combine the teaching of D1 and D2 to arrive at a method and composition as claimed, without investing any ingenuity. Thus the claimed subject-matter is also found to lack an inventive step.
Applicant's arguments presented in his letter of 11.06.2001 are not found to be convincing as the complexity of an organism does not necessarily protect it from sensitivity to a toxic substance and vice versa, therefore the objection against inventive step is upheld.

The requirements of Article 33 (1) and (3) PCT are thus not satisfied.

VII- Certain defects:

There are two claims 4, one of which pertains to fish (4, 1) and one which describes aphids (4,2). This should be amended.

The claims referring to organisms other than fish lice are not supported by the description, as no examples testing the effect of the claimed method and composition were carried out with aphids or head lice. Thus since these other "lice" are very different in nature and habitat and will require a completely different treatment from fish lice, it is doubtful whether the method and composition devised and tested for fish lice will work also for these other organisms. (See also unity objection).

The wording of claim 18 lacks any clear meaning as the "organism" to which it refers can mean anything beyond the scope of protection. Claim 11 must be deleted.

PATENT COOPERATION TREA

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION**	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
L/WZ42/cm/1		
International application No.	International filing date *(day month year)*	(Earliest) Priority Date *(day month year)*
PCT/NL 00/00196	23/03/2000	26/03/1999
Applicant		
CAMPINA MELKUNIE B.V. et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 2 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

[ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing:

[ ] contained in the international application in written form.

[ ] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readble form.

[ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I)

3. [ ] **Unity of invention is lacking** (see Box II)

4. With regard to the **title**,

[X] the text is approved as submitted by the applicant

[ ] the text has been established by this Authority to read as follows:

5. The figure of the **drawings** to be published with the abstract is Figure No.

[ ] as suggested by the applicant

[ ] None of the figures

Form PCT/ISA/210 (first sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/NL 00/00196

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A01N63/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, CHEM ABS Data, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 307 376 A (EWOS AB) 15 March 1989 (1989-03-15) claims 1-3 page 10, line 5 - line 11	11-17, 19,20
A	page 3, line 19	1-10,18
A	US 5 313 911 A (THOMASSEN JAN M ET AL) 24 May 1994 (1994-05-24) cited in the application	

[ ] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the

Date of the actual completion of the international search	Date of mailing of the international search report
21 June 2000	12/07/2000

Name and mailing address of the ISA	Authorized officer
	Decorte, S

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

[redacted]rmation on patent family members

[redacted]ternational Application No
PCT/NL 00/00196

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
EP 0307376	A	15-03-1989	AU	1871988 A	12-01-1989
			DK	381388 A	11-01-1989
			FI	883238 A	11-01-1989
			JP	1061427 A	08-03-1989
			NO	883059 A	11-01-1989
			SE	8702831 A	11-01-1989
US 5313911	A	24-05-1994	SE	468699 B	08-03-1993
			CA	2081218 A,C	25-04-1993
			DK	89692 A	21-08-1992
			GB	2260703 A,B	28-04-1993
			IE	69391 B	18-09-1996
			NO	178013 B	02-10-1995
			SE	9103113 A	08-03-1993

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark
Office, PCT
2011 South Clark Place Room
CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 27 February 2001 (27.02.01)	
International application No. PCT/NL00/00196	**Applicant's or agent's file reference** L/WZ42/cm/1
International filing date (day/month/year) 23 March 2000 (23.03.00)	**Priority date** (day/month/year) 26 March 1999 (26.03.99)

Applicant

KUSSENDRAGER, Klaas, Daniël et al

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

25 October 2000 (25.10.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

F. Zotomayor

Telephone No.: (41-22) 338.83.38